

CLAIMS

1. A method of producing a polypeptide having hexose oxidase activity, comprising isolating or synthesizing a DNA fragment encoding the polypeptide, introducing said DNA fragment into
5 an appropriate host organism in which the DNA fragment is combined with an appropriate expression signal for the DNA fragment, cultivating the host organism under conditions leading to expression of the hexose oxidase active polypeptide and recovering the polypeptide from the cultivation medium or from the host organism.

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2. A method according to claim 1 wherein the DNA fragment is isolated from a marine algal species.

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3. A method according to claim 2 wherein the marine algal species is one selected from the group consisting of *Chondrus crispus*, *Iridophycus flaccidum* and *Euthora cristata*.

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4. A method according to claim 1 wherein the host organism is a microorganism selected from the group consisting of a bacterial species, a fungal species and a yeast species.

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5. A method according to claim 4 wherein the host organism is selected from the group consisting of *E. coli*, *Saccharomyces cerevisiae* and *Pichia pastoris*.

6. A method according to claim 1 wherein the DNA fragment comprises at least one DNA sequence coding for an amino acid sequence selected from the group consisting of

(i) Tyr-Glu-Pro-Tyr-Gly-Gly-Val-Pro (SEQ ID NO:1),

(ii) Ala-Ile-Ile-Asn-Val-Thr-Gly-Leu-Val-Glu-Ser-Gly-Tyr-Asp-X-X-X-Gly-Tyr-X-Val-Ser-Ser (SEQ ID NO:2),

(iii) Asp-Leu-Pro-Met-Ser-Pro-Arg-Gly-Val-Ile-Ala-Ser-Asn-Leu-X-Phe (SEQ ID NO:3),

(iv) Asp-Ser-Glu-Gly-Asn-Asp-Gly-Glu-Leu-Phe-X-Ala-His-Thr (SEQ ID NO:4),

(v) Tyr-Tyr-Phe-Lys (SEQ ID NO:5),

(vi) Asp-Pro-Gly-Tyr-Ile-Val-Ile-Asp-Val-Asn-Ala-Gly-Thr-X-Asp (SEQ ID NO:6),

5 (vii) Leu-Gln-Tyr-Gln-Thr-Tyr-Trp-Gln-Glu-Glu-Asp (SEQ ID NO:7),

(viii) X-Ile-Arg-Asp-Phe-Tyr-Glu-Glu-Met (SEQ ID NO:8),

10 where X represents an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Asx, Cys, Gln, Glu, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val,

15 and muteins and variants hereof.

7. A method according to claim 1 which comprises as a further step a purification of the polypeptide preparation initially recovered from the cultivation medium and/or the microorganisms to obtain a preparation in which the polypeptide is in a substantially pure form.

15 8. A method according to claim 1 wherein the polypeptide having hexose oxidase activity is a fusion product.

9. A polypeptide in isolated form having hexose oxidase activity, comprising at least one amino acid sequence selected from the group consisting of

20 (i) Tyr-Glu-Pro-Tyr-Gly-Gly-Val-Pro (SEQ ID NO:1),

25 (ii) Ala-Ile-Ile-Asn-Val-Thr-Gly-Leu-Val-Glu-Ser-Gly-Tyr-Asp-X-X-X-Gly-Tyr-X-Val-Ser-Ser (SEQ ID NO:2),

(iii) Asp-Leu-Pro-Met-Ser-Pro-Arg-Gly-Val-Ile-Ala-Ser-Asn-Leu-X-Phe (SEQ ID NO:3),

(iv) Asp-Ser-Glu-Gly-Asn-Asp-Gly-Glu-Leu-Phe-X-Ala-His-Thr (SEQ ID NO:4),

5 (v) Tyr-Tyr-Phe-Lys (SEQ ID NO:5),

(vi) Asp-Pro-Gly-Tyr-Ile-Val-Ile-Asp-Val-Asn-Ala-Gly-Thr-X-Asp (SEQ ID NO:6),

(vii) Leu-Gln-Tyr-Gln-Thr-Tyr-Trp-Gln-Glu-Glu-Asp (SEQ ID NO:7),

10 (viii) X-Ile-Arg-Asp-Phe-Tyr-Glu-Glu-Met (SEQ ID NO:8),
where X represents an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Asx, Cys, Gln, Glu, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val,

15 and muteins and variants hereof.

10. A polypeptide according to claim 9 which is produced according to the method of claim 1.

11. A polypeptide according to claim 9 which is produced by a microbial cell selected from the group consisting of a bacterial cell, a fungal cell and a yeast cell.

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12. A polypeptide according to claim 11 which is produced by a cell selected from the group consisting of an *E. coli* cell, a *Saccharomyces cerevisiae* cell and a *Pichia pastoris* cell.

13. A polypeptide according to claim 9 which is in a substantially non-glycosylated form.

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14. A polypeptide according to claim 9 which has functional characteristics identical or partially identical to those of hexose oxidase naturally occurring in *Chondrus crispus*.

15. A polypeptide according to claim 14 which when subjected to SDS-PAGE shows separate bands of 29, 40 and/or 60 kD.

16. A polypeptide according to claim 9 which shows an enzymatic activity at a pH in the range of 5-9.

17. A polypeptide according to claim 9 which has an optimum temperature for enzymatic activity being in the range of 20-60°C.

18. A polypeptide according to claim 9 which oxidizes at least one sugar selected from the group consisting of D-glucose, D-galactose, maltose, cellobiose, lactose, D-mannose, D-fucose and D-xylose.

19. A polypeptide according to claim 9 which has an isoelectric point in the range of 4-5.

20. A polypeptide according to claim 19 which has an isoelectric point of 4.3 ± 0.1 .

21. A polypeptide according to claim 19 which has an isoelectric point of 4.5 ± 0.1 .

22. A polypeptide according to claim 9 which is in a substantially purified form.

23. A polypeptide according to claim 9 which has a molecular weight as determined by gel filtration using Sephadryl S-200 Superfine (Pharmacia) which is in the range of 100-150 kD,

24. A polypeptide according to claim 23 which has an apparent molecular weight of $110 \text{ kD} \pm 10 \text{ kD}$.

25. A polypeptide according to claim 9 which is part of a fusion product comprising additional enzymatically active amino acid sequences.

26. A recombinant DNA molecule comprising a DNA fragment 5 coding for a polypeptide having hexose oxidase activity.

27. A DNA molecule according to claim 26 wherein the DNA fragment codes for a polypeptide comprising at least one amino acid sequence as defined in claim 9, or a mutein or derivative of such polypeptide.

10 28. A DNA molecule according to claim 27 comprising the DNA sequence (SEQ ID NO:30):

TGAATTCTGTG GGTCGAAGA	CCCTTTGCCT CGTCTCTCTG GTACCGTGT	TGTCAAAGGT	60
TCGCTTGAC ACTGAACCTTC ACGATGGCTA CTCTTCCTCA GAAAGACCCC	GGTTATATTG	120	
TAATTGATGT CAACCGGGC ACCCGGGACA AGCCGGACCC	ACGTCTCCCC TCCATGAAGC	180	
AGGGCTTCAA CCGCCGCTGG ATTGGAACTA ATATCGATTT	CGTTTATGTC GTGTACACTC	240	
CTCAAGGTGC TTGTACTGCA CTTGACCGTG CTATGGAAA	GTGTTCTCCC GGTACAGTCA	300	
GGATCGTCTC TGGCGGCCAT TGCTACGAGG ACTTCGTATT	TGACGAATGC GTCAAGGCCA	360	
TCATCAACGT CACTGGTCTC GTTGAGAGTG GTTATGACGA CGATAGGGGT	TACTTCGTCA	420	
GCAGTGGAGA TACAAATTGG GGCTCCTTCA AGACCTTGT	T CAGAGACCAC GGAAGAGTTC	480	
TTCCCGGGGG TTCCTGCTAC TCCGTCGGCC TCGGTGGCCA	CATTGTGGC GGAGGTGACG	540	
GCATTTTGGC CCGCTTGCAT GGCTCCCCG TCGATTGGCT	CAGCGCGTG GAGGTCGTG	600	
TTAACGCCAGT CCTCACCGAA GACTCGGTAC TCAAGTATGT	GCACAAAGAT TCCGAAGGCA	660	
ACGACGGGGA GCTCTTTGG GCACACACAG GTGGCGGTGG CGGAAACTTT	GGAATCATCA	720	
CCAAATACTA CTTCAAGGAT TTGCCCATGT CTCCACGGGG	CGTCATCGCA TCAAATTTAC	780	
ACTTCAGCTG GGACGGTTTC ACGAGAGATG CCTTGCAGGA	TTTGTGACA AAGTACTTCA	840	
AACTTGCCAG ATGTGATTGG AAGAATACGG TTGGCAAGTT	TCAAATCTTC CATCAGGCAG	900	
CGGAAGAGTT TGTCAATGTAC TTGTATACAT CCTACTCGAA	CGACGCCGAG CGCGAAGTTG	960	
CCCAAGACCG TCACTATCAT TTGGAGGCTG ACATAGAACAA	GATCTACAAA ACATGCGAGC	1020	
CCACCAAAGC GCTTGGCGGG CATGCTGGGT GGGCGCCGTT	CCCCGTGCGG CCCGCGCAAGA	1080	

GGCACACATC	CAGACGTCG	TATATGCATG	ACGAGACGAT	GGACTACCCC	TTCTACGCGC	1140
TCAGTGGAGAC	GATCAACGGC	TCCGGGCCGA	ATCAGCGCGG	CAAGTACAAG	TCTGCGTACA	1200
TGATCARGGA	TTTCCCGGAT	TTCCAGATCG	ACGTGATCTG	GAAATACCTT	ACGGAGGTCC	1260
CGGACGGCTT	GACTAGTGCC	GAAATGAAGG	ATGCCTTA	CCAGGTGGAC	ATGTTTGGTG	1320
TGAGGATTCA	CAAGGTGGTC	TGGGATGCGA	CGGCAGTCGC	GCAGCGCGAG	TACATCATCA	1380
AACTGCAGTA	CCAGACATAC	TGGCAGGAAG	AAGACAAGGA	TGCAGTGAAC	CTCAAGTGGA	1440
TTAGAGACTT	TTACGAGGAG	ATGTATGAGC	CGTATGGCGG	GGTTCCAGAC	CCCAACACGC	1500
AGGTGGAGAG	TGGTAAAGGT	GTGTTGAGG	GATGCTACTT	CAACTACCCG	GATGTGGACT	1560
TGAACAACTG	GAAGAACGGC	AAGTATGGTG	CCCTCGAACT	TTACTTTTG	GGTAACCTGA	1620
ACCGCCTCAT	CAAGGCCAAA	TGGTTGTGGG	ATCCAACGA	GATCTTCACA	AACAAACAGA	1680
GCATCCCTAC	TAAACCTCTT	AAGGAGCCCA	AGCAGACGAA	ATAGTAGGTC	ACAATTAGTC	1740
ATCGACTGAA	GTGCAGCACT	TGTCGGATAC	GGCGTGATGG	TTGCTTTTA	TAAACTTGGT	1800
A						1801

29. A microbial cell which comprises the recombinant DNA molecule of claims 26.

30. A cell according to claim 29 which is selected from the group consisting of a bacterial cell, a fungal cell and a yeast cell.

31. A cell according to claim 30 which is selected from the group consisting of an *E. coli* cell, a lactic acid bacterial cell, a *Saccharomyces cerevisiae* cell and a *Pichia pastoris* cell.

10 32. A method of manufacturing a food product wherein a polypeptide according to claim 9 or a microbial cell according to claim 29 is used.

33. A method according to claim 32 wherein the food product is selected from the group consisting of a dairy product, a starch-containing food product and a non-dairy beverage.

34. A method according to claim 32 wherein the polypeptide is acting as an antimicrobial agent or as an antioxidant.

35. A method according to claim 32 wherein the polypeptide is acting as an oxygen removing agent in a food packaging.

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36. A method of manufacturing an animal feed wherein the polypeptide according to claim 9 or a microbial cell according to claim 29 is used.

37. A method according to claim 36 wherein the animal feed is
10 silage.

38. A method of reducing the sugar content of a food product, comprising adding to said product an amount of the polypeptide according to claims 9 or a microbial cell according to claim 29 which is sufficient to remove at least part
15 of the sugar initially present in said food product.

39. A method of manufacturing a product selected from the group consisting of a pharmaceutical product, a cosmetic and a tooth care product wherein a polypeptide according to claim 9 or a microbial cell according to claim 29 is used.

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40. A method of preparing a baked product from a dough, comprising adding the polypeptide according to claim 9 or a microorganism according to claims 29 capable of expressing such a polypeptide to the dough.

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41. A dough improving composition comprising a polypeptide according to claim 9 or a microorganism according to claim 29 capable of expressing such a polypeptide in dough, and at least one conventional dough component.

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42. A composition according to claim 41, further comprising at least one enzyme selected from the group consisting of a cellulase, a hemicellulase, a xylanase, a pentosanase, an amylase, a lipase and a protease.

43. An method of analyzing the content of a sugar in a sample wherein the polypeptide according to claim 9 or the microbial cell according to claims 29 is used as an analytical reagent.

5 44. A method of manufacturing a lactone using a polypeptide according to claim 9 or a microbial cell according to claims 29, said method comprising applying the polypeptide and/or the microbial cell to a reactor containing a carbohydrate which can be oxidized by the polypeptide and operating the

10 reactor under conditions where the carbohydrate is oxidized to a lactone.